

# Effect of Comorbid Anxiety Disorders on the Hypothalamic-Pituitary-Adrenal Axis Response to a Social Stressor in Major Depression

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**Background:** Major depressive disorder (MDD) is often complicated by anxiety symptoms, and anxiety disorders occur in approximately 30% of mood cases. This study examined the influence of anxiety comorbidity on the hypothalamic-pituitary-adrenal (HPA) axis response to stress in patients with MDD.

**Methods:** Untreated subjects with pure MDD ( $n = 15$ ), MDD with comorbid anxiety disorders ( $n = 18$ ), and pure anxiety disorders ( $n = 15$ ) were recruited by advertising. Age- and gender-matched control subjects were recruited for each subject with a psychiatric diagnosis ( $n = 48$ ). All subjects underwent a social stressor, the Trier Social Stress Test (TSST), and blood was collected for adrenocorticotrophic hormone (ACTH) and cortisol assay.

**Results:** When all depressed patients ( $n = 33$ ) were compared with their matched control subjects ( $n = 33$ ), they showed a significantly greater ACTH response to the stressor; however, this exaggerated ACTH response was exclusively due to the depressed group with comorbid anxiety disorders. A similar but nonsignificant effect was observed in the cortisol response. Subjects with pure mood or pure anxiety disorders showed normal ACTH and cortisol responses to the TSST. All patient groups showed similar levels of TSST-induced anxiety.

**Conclusions:** Comorbid anxiety disorders might play a role in the increased activation of the HPA axis observed in patients with major depression.

**Key Words:** ACTH, cortisol, stress, depression, anxiety disorders, social anxiety disorder

Comorbidity of mood and anxiety symptoms is a well-established finding. Many patients with major depression also have one or more anxiety disorders; many others have anxiety symptoms without meeting criteria for a specific disorder (Kessler et al 1994; Robins and Regier 1991). Substantial data suggest that depressed patients with anxiety diagnoses have worse depressive symptoms, a worse clinical course, higher risk of suicide, and possibly different family history (Brown et al 1996; Clayton 1990; Coryell et al 1992; Joffe et al 1993; Kara et al 2000; Kessler et al 1999; Lydiard 1991); however, the influence of comorbid anxiety disorders on the neuroendocrine picture of major depression has not been well studied. Neurobiological studies have historically focused on "pure" disorders, in the hopes that homogenous populations would more quickly reveal disease-specific pathophysiologic processes. Such work has been productive, suggesting, for example, that dysregulation within the hypothalamic-pituitary-adrenal (HPA) axis might play a central role in the pathophysiology of depression (Butler and Nemeroff 1990); however, the lack of more extensive attention to diagnostic and subsyndromal comorbidities might in fact have excluded from view important clues regarding the real links between syndromes, symptoms, and neuroendocrine functioning.

Studies of primary major depression have clearly demonstrated HPA axis dysregulation, revealing increased cortisol and adrenocorticotrophic hormone (ACTH) levels both at baseline and

after metyrapone, and defects in negative feedback, most commonly seen as escape from suppression in the dexamethasone suppression test (DST) (Carroll et al 1976, 1981; Halbreich et al 1985; Linkowski et al 1985; Pfohl et al 1985; Rubin et al 1987; Young et al 1994, 2001). Studies of primary anxiety disorders have revealed less robust HPA axis dysregulation (Abelson and Curtis 1996; Cameron et al 1987; Curtis et al 1982). In the DST, panic patients show rates of nonsuppression that are slightly elevated compared with normal subjects but much lower than those observed in major depression (Avery et al 1985; Coryell et al 1991; Curtis et al 1982; Uhde et al 1994; Westburg et al 1991). Patients with comorbid panic and depression show higher rates of nonsuppression than those with pure panic, comparable to the rates seen with pure depression (Avery et al 1985; Grunhaus 1994). A recent study demonstrated that patients with "mixed anxiety and depressive disorder" also show DST nonsuppression rates similar to those seen in major depression (Kara et al 2000). These data suggest that depression is more robustly linked to HPA axis dysregulation than is anxiety and "dominates" the neuroendocrine picture when the disorders are comorbid.

The situation might be different when depression and post-traumatic stress disorder (PTSD) are comorbid. Studies by Yehuda and colleagues in male combat veterans have shown that PTSD is associated with low cortisol and increased suppression of cortisol to dexamethasone, rather than the high cortisol and DST escape that is seen in depression (Yehuda 2002). When combat veterans or elderly holocaust survivors with comorbid PTSD and depression are examined, their neuroendocrine profile is not that of depression. Rather, they show the low baseline cortisol and enhanced negative feedback to dexamethasone associated with PTSD (Yehuda 2002; Yehuda et al 2002a, 2002b); however, there is substantial conflicting evidence (Heim et al 2000; Lemieux and Coe 1995; Rasmusson et al 2001; Young and Breslau 2004; Young et al 2004) regarding the neuroendocrine picture in premenopausal women with PTSD. A number of studies have observed increased baseline cortisol (Lemieux and Coe 1995; Maes et al 1998; Pittman and Orr 1990; Rasmusson et al 2001; Young and Breslau 2004). Furthermore, several studies

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have shown exaggerated stress response to both a psychosocial stressor and to exogenous corticotropin-releasing hormone (CRH) in women with comorbid PTSD and major depressive disorder (MDD) (Heim et al 2000; Rasmusson et al 2001). Consequently, even with PTSD the data are mixed, with several studies demonstrating greater activation of the HPA axis when PTSD and major depression are comorbid.

There are few studies of activation of the HPA axis by psychosocial challenge in either depression or anxiety. Initial work in major depression suggests a normal response to a public speaking stressor but with an apparent defect in the ability of high baseline cortisol to modulate the cortisol stress response (Young et al 2000; Heim et al 2000). This public speaking stress response has also been examined in social anxiety disorder, with one report of an increased cortisol response to the speaking challenge (Condren et al 2002), but other studies show a normal response (Furlan et al 2001; Gerra et al 2000; Levin et al 1993; Martel et al 1999).

Given the robust evidence for HPA axis dysregulation in depression and the relatively weak evidence for such dysregulation in anxiety disorders other than PTSD, the contribution of anxiety to the HPA axis dysregulation of depression might be expected to be minimal; however, there has been little direct, empirical examination of this issue except in the case of PTSD. It remains possible that there are undetected interactions between anxiety and depressive symptoms that contribute to neuroendocrine abnormalities in major depression. Given the extensive, subsyndromal overlap between anxiety and depressive symptoms and data suggesting shared underlying neurobiology (Butler and Nemeroff 1990) of depressive and anxiety symptoms even when comorbidity is excluded in an attempt to focus on supposedly "pure" disorders, the most direct approach to understanding symptom interactions might in fact be to begin including fully comorbid patients in our neuroendocrine studies rather than excluding them. Such work could help shed light on whether HPA axis abnormalities in depression are linked to disease-specific pathophysiologic processes, or might be linked to particular symptoms or symptom interactions that are independent of diagnosis, or might reflect a more general failure of coping mechanisms. The current studies were undertaken to address specifically the influence of comorbid anxiety on the HPA axis response to the Trier Social Stress Test (TSST), a standardized stressor involving public speaking and mental arithmetic. The design was to recruit subjects with "pure" anxiety disorders, "pure" major depression, and comorbid major depression and anxiety to compare the effects of comorbid anxiety of both symptom and syndromal levels on an HPA axis response to challenge in major depression.

## Methods and Materials

All studies were approved by the University of Michigan institutional review board. All subjects gave informed consent for a "public speaking task." Subjects with major depression and anxiety disorders and normal control subjects were recruited from the community through advertising, and all but one were outpatients at the time of the study. All subjects were free of any medication and medically healthy as verified by screening blood work and physical examination. After initial screening, the Structured Clinical Interview for DSM-IV was administered by a trained research nurse, and subjects meeting inclusion and exclusion criteria were recruited into the study. In addition to DSM-IV categorical ratings, we obtained dimensional ratings of

depression and anxiety, including the Hamilton Depression (Ham-D, 17 item) and Hamilton Anxiety (Ham-A) Rating Scales, the Spielberger trait anxiety scale, and Beck Depression Inventory, and also obtained the Global Assessment of Functioning and the Sheehan Disability Scale to measure impairment. The "pure" depression group was composed of subjects who had MDD and did not meet DSM-IV criteria for any current or past anxiety disorder. A "pure" anxiety group was composed of subjects who met DSM-IV criteria for an anxiety disorder (panic, generalized anxiety disorder, or social anxiety disorder), with no current or recent past depression. Subjects with obsessive-compulsive disorder were excluded. Posttraumatic stress disorder was neither an inclusion nor exclusion criterion. Four of the 15 subjects included in this group had a past episode of major depression, but these episodes had occurred a minimum of 2 years before the study, and in all cases the anxiety disorder predated the depressive episode. A comorbid group was composed of subjects who met criteria for either a current anxiety or depressive disorder and also met criteria within the past year for a disorder from the other category. Of the 18 subjects in the comorbid group, 17 had current anxiety disorders and one had panic disorder but was not currently experiencing panic attacks; all had current major depression at the time of study recruitment; however, in two subjects the episodes remitted between initial contact and completion of the study (2 months in remission). All subjects were free of all medication, including over-the-counter herbal remedies. Only five of the subjects had ever received psychotropic medications, and the most recent exposure was 9 months before the study. Normal subjects were individually matched for gender and age and were free of all psychiatric diagnoses, in themselves and their first-degree relatives.

## Challenge Procedures

On the day of the study, subjects reported to the University of Michigan Depression Program neuroendocrine study suite at 2:30 PM. They were placed in bed, and an intravenous catheter was inserted for blood drawing. Beginning at 3:00 PM, blood was drawn every 10 min for cortisol. At 3:30 PM, subjects were moved to another room for the TSST, a public speaking task involving a mock job interview and mental arithmetic (Kirschbaum et al 1993). At 4:00 PM, the panel came into the room and was introduced to the study subject as the panel for the task; at this point, the subject also received a complete description of the specific task (mock job interview). They were given 5 min to prepare a presentation in which they were to promote their candidacy for a job that was tailored to their interests. The task was also videotaped to increase task engagement. After 5 min for the job presentation, subjects were given a second unexpected task (i.e., to subtract 13 serially from 1022). They were told to stop and begin again whenever an error was made. This continued for another 5 min. Blood was drawn for ACTH and cortisol at 10-min intervals until 60 min after completion of the task. All samples were placed on ice immediately, and samples for ACTH were spun within 10 min of collection. In addition, on the day of the study, patients filled out state anxiety measures and rated the following emotions on a 100-mm visual analog scale: happy, anxious, nervous, calm, depressed, drowsy, sad, energetic, fearful, and angry. Ratings were collected immediately before the task description, at the end of the public speaking task, and 20 min after the challenge. Blood pressure and heart rate were recorded 30 min and immediately before the stressor and after completion of the challenge. All subjects participated in an additional clonidine challenge for measurement of growth hor-

**Table 1.** Characteristics of the Depression, Anxiety, and Control Groups

	Pure MDD	Comorbid MDD	Pure Anxiety	Control Subjects
No. of Subjects	15	18	15	48
Age (years)	28.7 ± 7.7	23.2 ± 5.5	25.4 ± 8.5	26.0 ± 7.1
Gender (Female/Male)	7/8	14/4	9/7	29/19
Ham-D Score <sup>a</sup>	19.1 ± 4 (SD)	12.7 ± 6.7	3.3 ± 3.4	.5 ± .9
Ham-A Score <sup>b</sup>	13.8 ± 5.5	12.4 ± 7.0	5.8 ± 7	.7 ± .7
Anxiety Diagnoses	0	18	15	0
Panic Disorder	0	5	3	0
Social Anxiety Disorder	0	13	12	0
PTSD	0	2 <sup>c</sup>	1 <sup>c</sup>	0

Data are presented as *n* or mean ± SD. MDD, major depressive disorder; Ham-D, Hamilton Depression Rating Scale; Ham-A, Hamilton Anxiety Rating Scale; PTSD, posttraumatic stress disorder.

<sup>a</sup> $F(3) = 137.9, p = .0001$  for group; by Fisher protected least significant difference (PLSD)  $p < .05$  for all group contrasts.

<sup>b</sup> $F(3) = 46.3, p = .001$  for group; by Fisher PLSD  $p < .05$  for control subjects vs. MDD, anxiety and comorbid; MDD vs. anxiety; and comorbid vs. anxiety.

<sup>c</sup>Also met criteria for panic disorder (1) and social anxiety disorder (1) in comorbid group and social anxiety disorder in anxiety group.

mone on a separate day at least 2 days apart (average 1 week, data reported separately). The order of the challenges was randomized.

### Hormone Assay

Cortisol was assayed with Coat-a-count kits (Diagnostic Products Corporation, Los Angeles, California). Adrenocorticotrophic hormone was assayed with Allegra HS immunoradiometric assay (Nichols Diagnostic Institute, San Juan Capistrano, California). Subjects and their matched control subjects were paired for all assays. Interassay variability for ACTH was 7.3% and for cortisol was 5%.

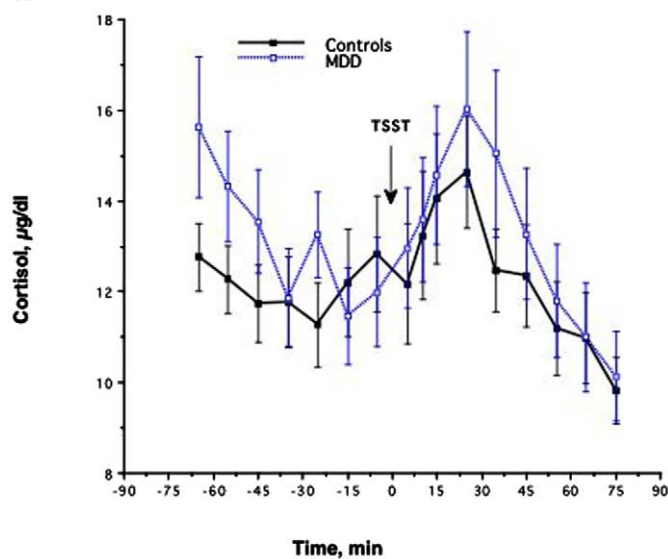
### Statistical Analyses

Data were analyzed with repeated-measures analyses of variance (ANOVA). Control subjects were recruited as specific matches for each patient, and only the specifically matched control subject–patient pairs were used in each ANOVA. This controls for age and gender differences, as well as assay variability. A *p* value of less than .05 was considered significant. Whether the TSST was administered before or after the clonidine challenge had no impact on the TSST results ( $p = .68$ ), so this factor was ignored in subsequent analyses.

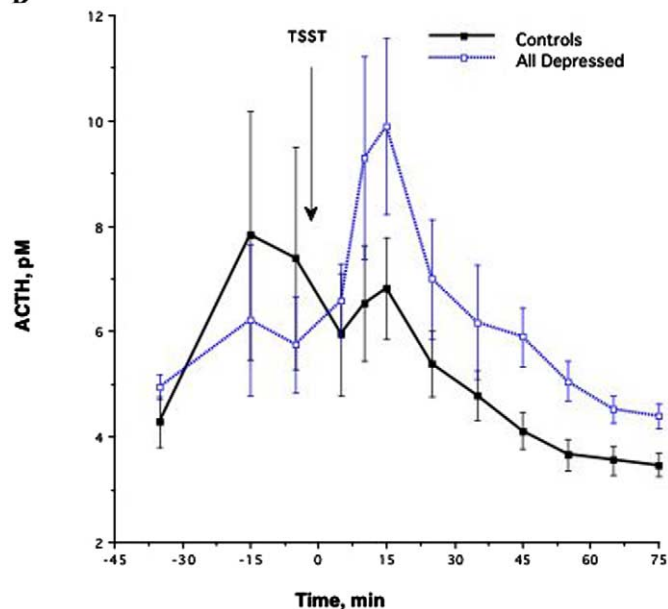
### Results

The study population is described in Table 1. The majority of comorbid subjects met criteria for social anxiety disorder, although five had panic disorder. Two of 15 pure depression subjects met criteria for dysthymia before the onset of MDD. By research diagnostic criteria, 9 of 15 pure MDD and 9 of 18 comorbid MDD subjects met criteria for probable or definite endogenous subtype; 7 of the pure depressed and 4 comorbid depressed subjects met DSM-IV criteria for melancholia. Three subjects (two pure depressed, one comorbid) met criteria for atypical depression. All patient groups demonstrated significantly higher Ham-D and Ham-A scores than control subjects (Table 1). All three patient groups demonstrated elevated Ham-A scores compared with control subjects, but the Ham-A scores in the pure anxiety group were less than those of the MDD group and comorbid group. Ham-D scores differed between groups

A



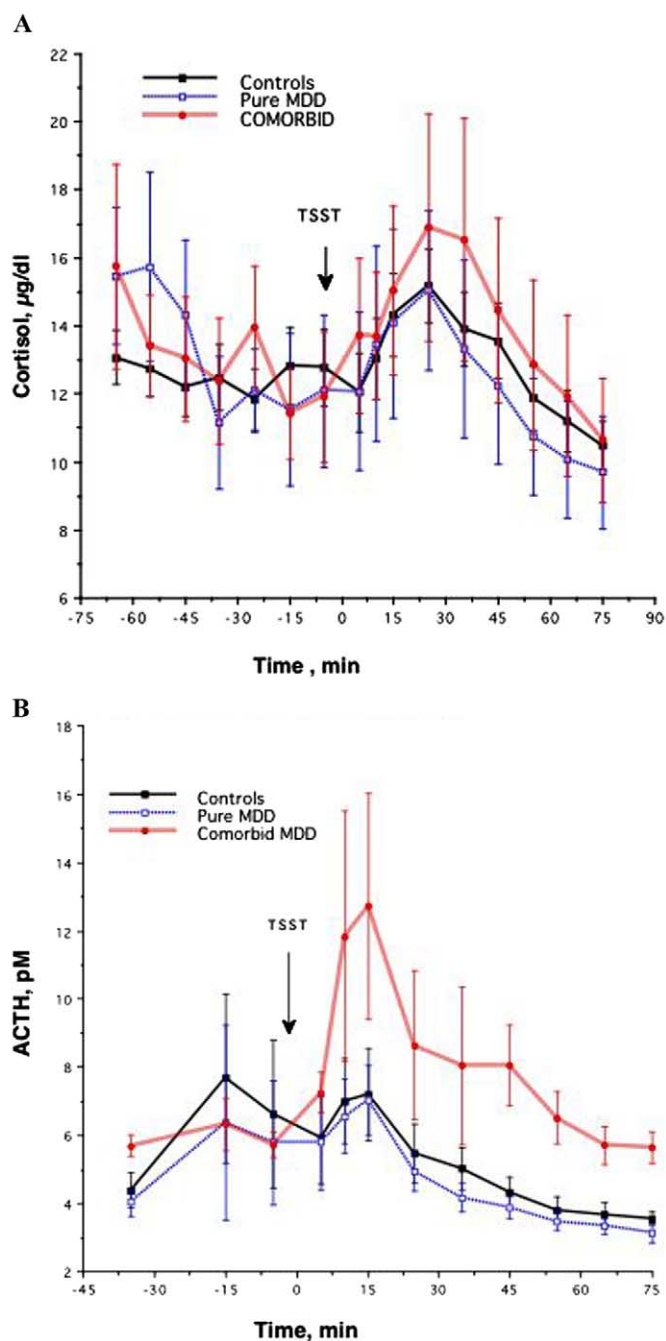
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**Figure 1.** (A) Cortisol and (B) adrenocorticotrophic hormone (ACTH) response ( $\pm$  SE) to the Trier Social Stress Test (TSST) in depressed patients and matched control subjects. A significantly greater ACTH response to the stressor is seen in the depressed subjects. The direction of the effect is the same for cortisol but did not reach significance. MDD, major depressive disorder.

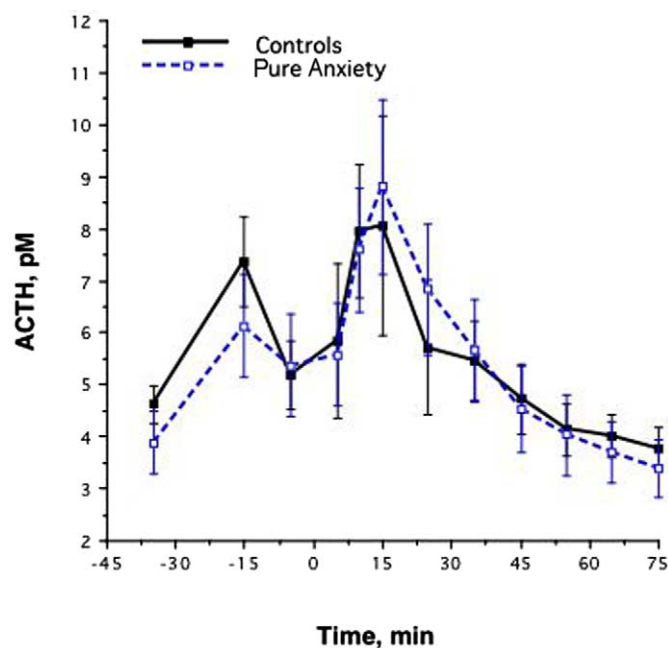
(Table 1), with the pure depressed group demonstrating significantly higher Ham-D scores than the comorbid group. The comorbid group showed higher Ham-D scores than the pure anxiety group.

Cortisol response data for all subjects are shown in Figure 1A. As in our previous study, depressed subjects did not differ from healthy control subjects in cortisol response to the TSST [repeated-measures ANOVA ( $-15$  to  $+75$  min) group  $F = .005, p = .9$ ; repeated-measures  $F(9) = 10.7, p = .0001$ ; interaction not significant]. Adrenocorticotrophic hormone data from the same subjects are shown in Figure 1B. As seen in the graph, a significant interaction effect indicates that depressed patients



**Figure 2.** (A) Cortisol and (B) adrenocorticotrophic hormone (ACTH) response ( $\pm$  SE) to the Trier Social Stress Test (TSST), separated by the presence or absence of an anxiety diagnosis in the depressed subjects. Only the depressed subjects with a comorbid anxiety diagnosis demonstrated increased ACTH response to the stressor. MDD, major depressive disorder.

demonstrated an elevated ACTH response to the TSST, relative to their matched control subjects [ $F(1) = 3.8, p = .539$  for group; repeated-measures  $F(10) = 8.46, p = .0001$ ; group  $\times$  repeated-measures interaction  $F(10) = 2.1, p = .02$ ]. Figure 2A shows the cortisol data for the depressed patients, split by anxiety subgroup. The comorbid group seems to show a greater cortisol response, but this did not reach statistical significance [group  $F = .131, p = .72$ ; repeated-measures  $F(9) = 4.8, p = .001$ ; interaction

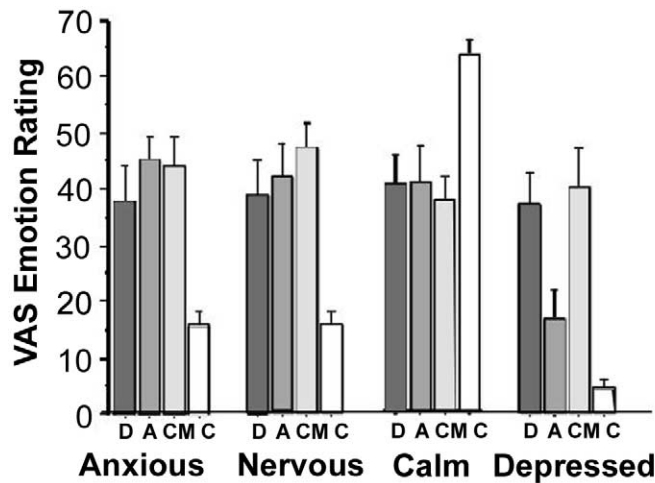


**Figure 3.** Adrenocorticotrophic hormone (ACTH) response ( $\pm$  SE) to the Trier Social Stress Test (TSST) in subjects with anxiety disorder only. Subjects with anxiety disorders without major depression demonstrated a normal response to the TSST.

$F(9) = 1.5, p = .15$ ]. In contrast, the ACTH data (Figure 2B) demonstrate a significantly greater response in the comorbid group compared with matched control subjects [ $F(1) = 1.67, p = .2$ ; repeated-measures  $F(11) = 3.72, p = .0001$ ; group  $\times$  repeated-measures interaction  $F(1,11) = 2.17, p = .016$ ]. The interaction effect remained significant when the comorbid group was compared with all control subjects [ $F(1,11) = 2.2, p = .012$ ]. Pure depressed patients did not differ from their matched control subjects [group  $F(1) = .375, p = .54$ ; repeated-measures  $F(10) = 4.95, p = .0001$ ; no significant interaction] or from all control subjects. Finally, the pure anxiety group (Figure 3) also showed a normal ACTH response to the TSST [ $F(1) = .007, p = .935$  for group; repeated-measures  $F = 9.523, p = .0001$ ; interaction not significant for either matched control subjects or all control subjects]. Their cortisol response was also normal [data not shown;  $F(1) = .4, p = .53$  for group; repeated-measures  $F = .84, p = .4$ ; interaction not significant].

Because there were gender differences in the composition of the groups, we examined whether there was an effect of gender on ACTH and cortisol measures. Overall, male subjects showed significantly higher baseline and postchallenge ACTH levels [ $F(1) = 12.56, p = .0007$ ; repeated-measures  $F(11) = 13.2, p = .0001$ ; interaction  $F(1,11) = 1.93, p = .0323$ ]. The mean difference between baseline and peak ACTH was 5.1 pmol/L for male subjects and 2.6 pmol/L for female subjects. Both patients and normal control subjects demonstrated similar gender differences [group  $F(1) = 4.96, p = .03$  for control subjects and  $F(1) = 7.2, p = .008$  for patients]. No gender difference was observed in cortisol measures.

We also collected blood pressure and heart rate data during the stressor. The pure depressed group had elevated heart rate throughout, but there were no other significant group differences. Subjective ratings of a number of emotions were obtained immediately before the stressor, at the end of the stressor, and 20



**Figure 4.** Mean ( $\pm$  SE) visual analog scale (VAS) ratings for feeling anxious, nervous, calm, and depressed over the course of the TSST in the four groups: pure depression (D), pure anxiety (A), comorbid (CM), and control subjects (C). Compared with control subjects, the three patient groups showed similarly elevated levels of anxiety/nervousness and reduced levels of calm. The two depressed groups reported more depressed feelings than did control subjects or pure anxiety patients. TSST, Trier Social Stress Test.

min later. Anxious distress was assessed by combining ratings of anxiety, nervousness, and calm (scale reversed for analysis). Anxious distress was slightly higher at the end of the stressor than at the beginning and had fallen below initial levels 20 min later. The patient groups all endorsed greater anxious distress than control subjects throughout (group  $F$ s all  $> 13.1$ ,  $p < .0001$ ) and did not differ from each other ( $p > .55$  for all variables). The pure and comorbid depressed groups had higher visual analog scale depression ratings than control subjects and the pure anxiety group throughout the procedure (group  $F = 23.8$ ,  $p < .0001$ ) but did not differ from each other ( $p = .73$ ). Visual analog scale data are presented in Figure 4.

We also explored whether dimensional ratings would better predict the overall ACTH response to the TSST than categorical diagnoses based on DSM-IV. We tried two approaches. One was to divide subjects on the basis of Ham-A scores into high ( $>15$ ) and low ( $<10$ ) anxiety. For the analysis of subjects with high Ham-A scores, we had a total of 10 subjects and matched control subjects. There was no difference between these subjects and their matched control subjects [ $F(1) = .28$ ,  $p = .6$ ; repeated-measures  $F(11) = 2.58$ ,  $p = .004$ ; interaction  $F = .876$ ,  $p = .56$ ]. Similarly, subjects with low Ham-A scores ( $n = 19$ ) did not differ from their matched control subjects [ $F(1) = .051$ ,  $p = .8$ ; repeated-measures  $F(11) = 8.15$ ,  $p = .0001$ ; interaction  $F(1,11) = 1.29$ ,  $p = .2$ ]. We also looked to see whether there was a correlation between Ham-A score and log-transformed ACTH response (lnAUC ACTH). The  $r^2$  was .03, a nonsignificant relationship ( $p = .255$ ). We examined the relationship between trait anxiety and lnAUC ACTH and found no relationship ( $r^2 = .000009$ ,  $p = .985$ ). We further examined the relationship between impairment as defined by the Sheehan Disability Measure and found no relationship to the lnAUC ACTH ( $r^2 = .004$ ,  $p = .69$ ). Consequently, it seems that diagnosis, rather than dimensional ratings of anxiety or impairment, shows the strongest relationship to increased stress reactivity.

We also examined the impact of depression severity on ACTH response to the TSST, by dividing patients according to Ham-D

scores. Subjects with a Ham-D score of 20 or greater ( $n = 10$ ) did not differ from either their matched control subjects or all control subjects. Similarly, subjects with a Ham-D score of less than 15 ( $n = 25$ ) did not differ from either matched control subjects or all control subjects in their ACTH or cortisol responses to the TSST.

## Discussion

These data demonstrate an increased ACTH response to the TSST in depressed patients, but this exaggerated endocrine response to the psychosocial stressor was only seen in depressed patients who also had a comorbid anxiety disorder. The "pure" depression group had a normal response. The increased ACTH response in the comorbid group was not associated with a significantly greater cortisol response, although the cortisol data do show the same direction of effect. The impact of comorbidity on the ACTH response seemed to be a function of having an anxiety disorder and not merely being anxious, because the pure depressed group had Ham-A scores that were as high as those seen in the comorbid group, and high anxiety itself was not associated with an increased ACTH response in dimensional analyses. Furthermore, all three patient groups had identically elevated levels of anxious distress during the stressor itself; and the depressed and the comorbid groups had identically elevated self ratings of depression at the time of the stressor. These findings support the idea that whatever aspect of comorbidity created the comorbid group's increased ACTH response to the stressor, it was not a function of heightened anxiety or depression in response to the challenge itself. It was also unlikely due to mere presence of an anxiety disorder, because this same increase was not observed in the pure anxiety group. The elevated ACTH response to challenge seems to be associated with the interactive presence of both depression and an anxiety disorder, and not merely with the presence of specific symptoms.

Several points in the data require further comment. One is that we saw a high degree of variability in the endocrine response to the TSST, with a less robust overall response to the stressor than expected. Despite this variability, a highly significant effect of time was observed in all analyses, with a rise in ACTH and cortisol levels after the stressor observed in all groups, indicating that the stressor did impact enough subjects to produce an overall effect. In another TSST study conducted with saliva cortisol in an undergraduate population, we also found that a substantial number of subjects demonstrated no response to the stressor (Young and Nolen-Hoeksema 2001). It is possible that had the overall endocrine response to the challenge been more robust, the pattern of group differences detected might have been altered.

A second point requiring comment involves the low Ham-A ratings in the pure anxiety group. This might raise questions as to how ill these patients actually were; however, the majority of patients in this group had social anxiety disorder, and the Ham-A, which focuses on general psychic and physical anxiety, does not well capture the specific distress of this disorder. These patients might in fact be expected to be particularly sensitive to the public performance challenge used, regardless of Ham-A scores.

A third point is the low Ham-D ratings in the comorbid group, especially because comorbidity is generally associated with greater severity. In part, this occurred because two individuals who had recently recovered from depression were included in this group; however, even when these individuals were excluded Ham-D scores were lower in this group. Although a more

severely affected group with both disorders might have been better, the inclusion of the less severely depressed subjects should have biased against finding an effect of comorbidity. We in fact found a greater response to the TSST in this comorbid group, even though they were significantly less depressed than the pure depression group on the Ham-D, and the two groups endorsed identical levels of depressed mood during the challenge itself.

The gender distribution was markedly different in the pure depressed compared with the comorbid group. Whereas the pure depressed group showed a 1:1 ratio of women to men, the comorbid group showed a 3:1 ratio. In part, this derives from the epidemiology of the disorders (i.e., the excess of women with mood and anxiety disorders; Kessler et al 1999). It is possible that gender influenced our results, but both our own data and data from Hellhammer's group show that women demonstrate smaller ACTH responses to the TSST than men (Kirschbaum et al 1999). Because our comorbid group had the greatest ratio of women to men, these gender differences cannot explain the larger ACTH response that we found in that group. Finally, we should acknowledge the small number of subjects studied and the need for replication of these studies.

The current data agree with our previous study showing a normal cortisol response to the TSST in major depression (Young et al 2000). In that study, we only included two subjects with comorbid anxiety diagnoses (primary MDD by research diagnostic criteria), so no conclusion could be drawn regarding comorbidity. Inclusion of a comorbid major depression and anxiety group as well as pure major depression and pure anxiety groups in this study demonstrated an increased response to this stressor in the comorbid group only. The predominant diagnosis in the comorbid group was social anxiety disorder. It is possible that the augmented response seen in this group resulted from the unique sensitivity of social phobics to a public speaking stressor; however, ACTH and cortisol responses to the public speaking task in our pure anxiety group (which had even more cases of social anxiety disorder) was normal, in agreement with three other reports on public speaking tasks in social anxiety disorders (Furlan et al 2001; Gerra et al 2000; Levin et al 1993; Martel et al 1999). In addition, the levels of emotional reactivity to the challenge did not differ between the three patient groups in this study. It thus does not seem that the specific anxiety about public performance associated with social anxiety disorder, by itself, can account for the increased reactivity to the TSST in our comorbid depressed and anxious group. The data suggest that the comorbid patients differ in response to this stressor from patients with either pure depression or a pure anxiety disorder and support the idea that future neurobiological studies should specifically examine such groups rather than exclude them. Intriguingly, the same biological effect of comorbid mood and anxiety disorders has been reported in a study with MDD patients, in which an exaggerated ACTH response to the TSST was also seen in women who had comorbid PTSD that resulted from childhood abuse (Heim et al 2000). One possibility is that the neurobiology of depression results in an increased capacity of the HPA axis to respond to provocative stimuli and that the addition of comorbid social anxiety disorder provided the salience for the particular probe we are using. Future studies with other types of challenges will be necessary to determine to generalizability of these findings to other HPA axis challenges.

Previous studies of comorbid anxiety and depression have used the DST to focus on the negative feedback component of the HPA axis and have shown that comorbid patients look like

pure depressives when this type of probe is used (Grunhaus 1994). Existing data clearly demonstrate reduced negative feedback in pure, primary MDD, which is also likely present in comorbid depression and panic (Avery et al 1985; Grunhaus 1994). The present study could not definitely determine whether our comorbid group has a similar defect in negative feedback, because the TSST does not specifically probe the feedback system; however, our data suggest that they do differ from patients with MDD alone in an activational component of HPA axis control. Further work applying both activational and feedback probes to patients with pure and comorbid disorders is needed to clarify this picture.

One reason to have expected that our comorbid depression and anxiety group would show greater HPA axis activation is that comorbidity is generally associated with greater depression severity, and HPA axis dysregulation has been linked to severity (Dratcu and Calil 1989; Kumar et al 1986; Osuch et al 2001); however, in our data the comorbid group actually had less severe depression, as measured by the Ham-D. Dimensional analyses also failed to demonstrate a link between depression severity and HPA response. Thus, depression severity cannot provide an explanation for the greater HPA axis activation seen in the comorbid group. An alternative explanation links hypotheses about the underlying neurobiology of mood and anxiety disorders and the potential interacting roles of CRH and noradrenergic systems in these disorders. Corticotropin-releasing hormone is well known as the activator of the HPA axis, but extrahypothalamic CRH also plays a role in anxiety symptoms (Butler and Nemeroff 1990; Dunn and Berridge 1990; Koob et al 1993). Injection of CRH into the brain is anxiogenic in a number of species (Butler and Nemeroff 1990; Dunn and Berridge 1990; Koob et al 1993). Furthermore, noradrenergic output from the locus coeruleus (LC) can activate central CRH systems and the HPA axis, as well as autonomic systems (Plotsky 1987; Plotsky et al 1989). Excessive noradrenergic output from the LC is thought to play a role in the pathophysiology of anxiety disorders (Bremner et al 1996; Charney and Heninger 1985; Charney and Redmond 1983). Individuals with comorbid anxiety and depression might have increased activity in both noradrenergic (anxiety) and CRH systems (depression) and thus might show greater reactivity of the HPA axis than individuals with hyperactivity of the CRH system alone. This agrees with previous data demonstrating a correlation between cerebrospinal fluid norepinephrine and indices of HPA axis activation in major depression (Rosenbaum et al 1983; Roy et al 1987; Wong et al 2000). Further research on the HPA axis in comorbid depression and anxiety disorders, with inclusion of a broader group of comorbid anxiety disorders, is necessary to better understand how different neuroendocrine stress response systems interact in controlling "final" outputs and in shaping stress sensitivity.

In conclusion, the present study demonstrated a greater ACTH response to a social stressor in patients with major depression; however, this increased response is due to the subgroup with comorbid anxiety disorders, because the depressed group without comorbid anxiety showed a normal ACTH and cortisol response to the TSST. These data suggest the need for further research on the influence of comorbid anxiety disorders on HPA axis function in major depression. Understanding more about the underlying pathophysiology of comorbidity might aid in the design of new treatments for the comorbid mood and anxiety syndromes and could help identify biological markers to define endophenotypes for future genetic linkage studies.

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